Supporting Information

for

Exploring the scope of DBU-promoted amidations of 7-methoxycarbonylppterin

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General procedures, characterization data, and copies of NMR spectra
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General information:
All reagents used were of commercial quality and obtained from Aldrich Chemical Co. and used as received. $^1$H and $^{13}$C NMR spectra were recorded in DMSO-$d_6$ (or methanol-$d_4$ for NMR kinetics) with a Bruker spectrometer using the solvent as the reference. Chemical shifts are given in parts per million (ppm). IR data were collected with a NEXUS 670 FT-IR. Purity was assessed on a Waters UPLC with a QDa detector. High resolution mass spectrometry was performed with a Varian 9.4T QFT-ESI ICR system. Filtrations were performed by aid of reduced pressure. Amidation reactions were run using an Anton-Paar MonoWave50$^\text{TM}$ reactor, operated without microwave irradiation and simply worked as a controlled heating block.¹

Synthesis

7-Methoxycarbonylpterin (1): Compound 1 was prepared by our previously reported acyl-radical insertion reaction.²

General method for the DBU-assisted amidation:
Compound 1 (50 mg, 0.23 mmol) was suspended in 1.0 mL of anhydrous MeOH in an oven-dried borosilicate reaction vessel (Anton-Paar), and stirred to create a slurry. To this was added DBU (68 µL, 0.45 mmol, 2 equiv) which resulted in full dissolution of 1. To this was added the amine (2 equiv). (Note: for amines containing an acidic functional group, e.g. Gly/Ser/Ala, 4 equiv DBU were used to ensure basic conditions). The reaction vessel was then fitted with a silicone cap containing a PTFE seal. The tube was placed in a MonoWave50$^\text{TM}$ reactor and irradiated for the time and temperature indicated. Once the reaction was complete, the mixture was diluted with 1 mL of DI water, and acidified by the dropwise addition of 3 M HCl, resulting in a yellow precipitate. The product was isolated by filtration, rinsed several times with water and MeOH, and dried over P$_2$O$_5$ in a vacuum oven.

(2-Amino-4-oxo-3,4-dihydropteridine-7-carbonyl)glycine (2): Compound 2 was synthesized by the general method above, using 34 mg glycine and 4 equiv DBU, resulting in 59 mg (99%) 2. MP > 300°C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$(ppm)= 12.72 (br, 1H),
11.61 (s, 1H), 9.04 (t, J = 5.6 Hz, 1H), 8.88 (s, 1H), 7.03 (br, 2H), 3.99 (d, J = 6.0 Hz); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) (ppm)= 171.2, 163.5, 160.8, 156.5, 154.9, 147.6, 136.9, 132.4, 41.5; HRMS-ESI (m/z) [M–H]\(^+\) calc. for (C\(_9\)H\(_7\)N\(_6\)O\(_4\))\(^-\) 263.0534; found 263.0533. This is consistent with that reported in the literature.\(^3\)

**2-Amino-N-(4-nitrophenethyl)-4-oxo-3,4-dihydropoteridine-7-carboxamide (3):**

Compound 3 was synthesized by the general method, using 91 mg of 4-nitrophenethylamine hydrochloride and 4 equiv DBU, resulting in 77 mg (96%) of **3.** MP > 300\(^\circ\)C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) (ppm)= 11.58 (s, 1H), 8.98 (t, J = 6 Hz, 1H), 8.83 (s, 1H), 8.15 (d, J = 8.8 Hz, 2H), 7.54 (d, J = 8.8 Hz, 2H), 6.98 (br, 2H), 3.62 (q, J = 6.8 Hz, 2H), 3.04 (t, J = 7 Hz, 2H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) (ppm)= 163.3, 160.8, 156.5, 154.8, 148.3, 148.1, 146.6, 137.0, 132.1, 130.5, 123.9, 40.8, 35.1; IR (cm\(^{-1}\)): 3414, 3216, 1722, 1637, 1514, 1342; HRMS-ESI (m/z) [M+H]\(^+\) calc. for (C\(_{15}\)H\(_{14}\)N\(_7\)O\(_4\))\(^+\) 356.1102; found 356.1101

**2-Amino-N-benzyl-4-oxo-3,4-dihydropoteridine-7-carboxamide (4):** Compound 4 was synthesized by the general method, using 50 \(\mu\)L of benzylamine and 2 equiv DBU, resulting in 64 mg (91%) of **4.** MP > 300\(^\circ\)C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) (ppm)= 11.63 (s, 1H), 9.41 (t, J = 6.3 Hz, 1H), 8.88 (s, 1H), 7.35-7.2 (m, 5H), 7.01 (br, 2H), 4.57 (d, J = 6 Hz, 2H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) (ppm)= 163.4, 160.8, 156.6, 154.8, 148.3, 139.6, 137.2, 132.1, 128.7 (2C), 127.8 (2C), 127.3, 42.9; IR (cm\(^{-1}\)): 3434, 3321, 3189, 1713, 1635, 1518, 1222, 772; HRMS-ESI (m/z) [M+H]\(^+\) calc. for (C\(_{14}\)H\(_{13}\)N\(_5\)O\(_2\))\(^+\) 297.1094; found 297.1095.

**2-Amino-4-oxo-3,4-dihydropoteridine-7-carboxamide (5):** Compound 5 was synthesized by the general method above, using 48 mg serine and 4 equiv DBU, resulting in 58 mg (88%) of **5.** MP > 300\(^\circ\)C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) (ppm)= 13.02 (br, 1H), 11.65 (s, 1H), 8.89 (s, 1H), 8.68 (d, J = 7.9 Hz, 1H), 7.05 (br, 2H), 5.27 (br, s, 1H), 4.50 (dt, J = 7.9, 3.6 Hz, 1H), 3.93-3.79 (m, 2H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) (ppm)= 171.9, 162.7, 160.8, 156.4, 155.0, 147.2, 136.7, 132.6, 61.5, 55.2; HRMS-ESI (m/z) [M–H]\(^+\) calc. for (C\(_{10}\)H\(_{9}\)N\(_5\)O\(_3\))\(^-\) 293.0640; found 293.0638. This is consistent with that reported in the literature.\(^3\)

**2-Amino-4-oxo-N-propyl-3,4-dihydropoteridine-7-carboxamide (6):** Compound 6 was synthesized by the general method, using 37 \(\mu\)L of 1-propylamine and 2 equiv DBU, resulting in 49 mg (87%) of **6.** MP > 300\(^\circ\)C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) (ppm)= 11.68 (s, 1H), 8.86 (s, 1H), 8.82 (t, J = 6 Hz, 1H), 7.05 (br, 2H), 3.28 (q, J = 7.2 Hz, 2H), 1.57 (sext., J = 7.2 Hz, 2H), 0.88 (t, J = 7.2 Hz, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) (ppm)= 163.2, 160.9, 156.3, 154.8, 148.4, 137.1, 132.0, 41.1, 22.8, 11.8; IR (cm\(^{-1}\)): 3260, 3130, 2964, 1705, 1644, 1518, 1397; HRMS-ESI (m/z) [M+H]\(^+\) calc. for (C\(_{10}\)H\(_{13}\)N\(_5\)O\(_3\))\(^+\) 249.1094; found 249.1095.

**2-Amino-N-(2-hydroxypropyl)-4-oxo-3,4-dihydropoteridine-7-carboxamide (7):**

Compound 7 was synthesized by the general method, using 35 \(\mu\)L of 1-aminom-2-propanol and 2 equiv DBU, resulting in 51 mg (85%) of **7.** MP > 300\(^\circ\)C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) (ppm)= 11.59 (s, 1H), 8.87 (s, 1H), 8.61 (t, J = 5.6 Hz, 1H), 7.02 (br, 2H), 4.88 (d, J = 4.8 Hz, 1H), 3.82 (quint., J = 6.4 Hz, 1H), 3.36 (m, 1H), 3.21 (m, 1H), 1.08 (d, J = 6.4 Hz, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) (ppm)= 163.0, 160.9, 156.4, 154.9, 148.0, 136.9, 132.2, 65.3, 46.9, 21.6; IR (cm\(^{-1}\)): 3400-3100 (broad/overlapping), 3093, 1728, 1679, 1631, 1518, 1230; HRMS-ESI (m/z) [M+H]\(^+\) calc. for (C\(_{10}\)H\(_{13}\)N\(_6\)O\(_3\))\(^+\) 265.1044; found 265.1043.
2-Amino-N-(4-aminophenethyl)-4-oxo-3,4-dihydropteridine-7-carboxamide (8): Compound 8 was synthesized by the general method above, using 59 µL 4-(2-aminoethyl)aniline and 2 equiv DBU, which provided 56 mg (76%) of 8. mp > 300°C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\)(ppm)= 11.59 (s, 1H), 8.85 (s, 1H), 8.74 (t, \(J = 5.6\) Hz, 1H), 7.03 (br, 2H), 6.89 (d, \(J = 8\) Hz, 2H), 6.51 (d, \(J = 8\) Hz, 2H), 5.46 (br, 2H), 3.47 (q, \(J = 6.8\) Hz, 2H), 2.69 (t, \(J = 7.2\) Hz, 2H); \(^13\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\)(ppm)= 162.9, 160.8, 154.9, 148.2, 146.9, 137.0, 132.1, 129.4 (2C), 126.7, 121.2, 114.7 (2C), 41.3, 34.7; IR (cm\(^{-1}\)): 3413, 3340, 3195, 1731, 1655, 1530, 1426; HRMS-ESI (m/z) [M+H]\(^+\) calc. for (C\(_{15}\)H\(_{16}\)N\(_7\)O\(_2\))\(^+\) 326.1360; found 326.1358

2-Amino-N-(2-hydroxyethyl)-N-methyl-4-oxo-3,4-dihydropteridine-7-carboxamide (9): Compound 9 was synthesized by the general method above, using 37 µL \(N\)-methylaminoethanol and 2 equiv DBU. This resulted in 43 mg (71%) of 9 which exists as a 2:1 mixture of the \(s\)-cis and \(s\)-trans rotamers. MP > 300°C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\)(ppm)= \{two rotamers\}\(^4\) 8.41 (s, 1H) [8.45 minor rotamer], 7.07 (br, 2H) \{overlapping minor rotamer\}, 4.71 (t, \(J = 5.2\) Hz, 1H) [4.84 minor rotamer], 3.51 (q, \(J = 5.2\) Hz, 2H) [3.65 minor rotamer], 3.41 (t, \(J = 5.6\) Hz, 2H) [3.55 minor rotamer], 3.05 (s, 3H) [3.01 minor rotamer]; \(^13\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\)(ppm)= \{two rotamers\} 167.0 [166.2 minor rotamer], 161.2, 156.2, 155.1, 154.1 [153.9 minor rotamer], 138.3 [137.6 minor rotamer], 129.5 [129.8 minor rotamer], 58.8 [58.7 minor rotamer], 52.5 [50.2 minor rotamer], 33.6 [37.9 minor rotamer]; IR (cm\(^{-1}\)): 3395, 3259, 2734, 1697, 1613, 1520, 1405, 1234; HRMS-ESI (m/z) [M+H]\(^+\) calc. for (C\(_{10}\)H\(_{13}\)N\(_6\)O\(_3\))\(^+\) 265.1044; found 265.1044

2-Amino-4-oxo-N-(2-(pyridin-2-yl)ethyl)-3,4-dihydropteridine-7-carboxamide (10): Compound 10 was synthesized by the general method, using 54 µL 2-(2-aminoethyl)pyridine and 2 equiv DBU, which provided 46 mg (66%) of 10. mp > 300°C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\)(ppm)= 11.63 (s, 1H), 8.95 (t, \(J = 6\) Hz, 1H), 8.85 (s, 1H), 8.54 (d, \(J = 4\) Hz, 1H), 7.78 (t, \(J = 7.2\) Hz, 1H), 7.35 (d, \(J = 7.6\) Hz, 1H), 7.29 (t, \(J = 6\) Hz, 1H), 7.02 (br, 2H), 3.71 (q, \(J = 6.4\) Hz, 2H), 3.07 (t, \(J = 6.8\) Hz, 2H); \(^13\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\)(ppm)= 165.5, 163.2, 160.9, 159.1, 154.8, 148.8, 148.1, 137.9, 137.0, 132.1, 124.0, 122.3, 39.1, 37.0; IR (cm\(^{-1}\)): 3126, 2973, 1672, 1609, 1436, 1134, 767; HRMS-ESI (m/z) [M+H]\(^+\) calc. for (C\(_{14}\)H\(_{14}\)N\(_6\)O\(_2\))\(^+\) 312.1203; found 312.1203

2-Amino-N-isopropyl-4-oxo-3,4-dihydropteridine-7-carboxamide (11): Compound 11 was synthesized by the general method, using 40 µL of 2-propylamine and 2 equiv DBU, resulting in 37 mg (66%) of 11. MP > 300°C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\)(ppm)= 11.59 (s, 1H), 8.85 (s, 1H), 8.48 (d, \(J = 8\) Hz, 1H), 6.98 (br, 2H), 4.14 (oct, \(J = 6.8\) Hz, 1H), 1.21 (d, \(J = 6.4\) Hz, 6H); \(^13\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\)(ppm)= 165.5, 162.3, 160.9, 154.8, 148.5, 137.1, 131.9, 41.4, 22.5 (2C); IR (cm\(^{-1}\)): 3141, 2974, 1695, 1633, 1515, 1386; HRMS-ESI (m/z) [M+H]\(^+\) calc. for (C\(_{10}\)H\(_{14}\)N\(_6\)O\(_2\))\(^+\) 249.1094; found 249.1093

2-Amino-4-oxo-N-(1-phenylethyl)-3,4-dihydropteridine-7-carboxamide (12): Compound 12 was synthesized by the general method, using 59 µL of \(\alpha\)-methylbenzylamine and 2 equiv DBU, resulting in 42 mg (60%) of 12. MP > 300°C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\)(ppm)= 11.65 (s, 1H), 9.07 (d, \(J = 8.4\) Hz, 1H), 8.83 (s, 1H), 7.44 (d, \(J = 7.2\) Hz, 2H), 7.34 (t, \(J = 7.2\) Hz, 2H), 7.25 (t, \(J = 7.2\) Hz, 1H), 7.03 (br, 2H), 5.19 (quint, \(J = 7.6\) Hz, 1H), 1.54 (d, \(J = 7.2\) Hz, 3H); \(^13\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\)(ppm)= 162.6, 161.0, 156.5, 154.9, 148.4, 144.5, 137.2, 132.1, 128.8 (2C), 127.3, 126.7 (2C), 48.9,
22.3; IR (cm⁻¹): 3405, 3141, 1643, 1515, 1397, 773, 693; HRMS-ESI (m/z) [M+H]+ calc. for (C₁₅H₁₅N₂O₄)⁺ 311.1251; found 311.1252

(2-Amino-4-oxo-3,4-dihydropteridine-7-carbonyl)alanine (13): Compound 13 was synthesized by the general method above, using 41 mg alanine and 4 equiv DBU, resulting in 31 mg (49%) 13. MP > 300°C. ¹H NMR (400 MHz, DMSO-d₆) δ(ppm)= 12.90 (br, 1H), 11.67 (s, 1H), 8.86 (s, 1H), 8.83 (d, J = 8 Hz, 1H), 7.04 (br, 2H), 4.48 (quint, J = 7.2 Hz, 1H), 1.45 (d, J = 7.2 Hz, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ(ppm)= 173.9, 165.6, 162.8, 160.8, 154.9, 147.6, 136.9, 132.4, 48.3, 17.7; HRMS-ESI (m/z) [M–H]⁻ calc. for (C₁₀H₈N₂O₄)⁻ 277.0691; found 277.0689. This is consistent with that reported in the literature.³

Methyl 5-((2-amino-4-oxo-3,4-dihydropteridine-7-carboxamido)methyl)furane-2-carboxylate (14): Compound 14 was synthesized by the general method above, using 69 mg methyl 5-(aminomethyl)-furan-2-carboxylate⁵ and 2 equiv DBU. This resulted in a mixture of the product and 7-carboxy-pterin, which was sonicated in a dilute aqueous solution of NaHCO₃ to dissolve the acid and the product was isolated by filtration, resulting in 37 mg (47%) 14 after drying. MP > 300°C. ¹H NMR (400 MHz, DMSO-d₆) δ(ppm)= 11.62 (s, 1H), 9.45 (t, J = 6 Hz, 1H), 8.88 (s, 1H), 7.24 (d, J = 3.6 Hz, 1H), 7.02 (br, 2H), 6.5 (d, J = 3.2 Hz, 1H), 4.56 (d, J = 6 Hz, 2H), 3.79 (s, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ(ppm)= 163.5, 160.8, 158.7, 157.4, 156.2, 154.8, 147.9, 143.2, 137.2, 132.3, 119.8, 110.0, 52.1, 36.7; IR (cm⁻¹): 3126, 1681, 1518, 1338, 1204, 1016, 761 HRMS-ESI (m/z) [M+H]+ calc. for (C₁₄H₁₃N₂O₃)⁺ 345.0942; found 345.0942

2-Amino-7-(morpholine-4-carboxy)pteridin-4(3H)-one (15): Compound 15 was synthesized by the general method above, using 40 µL morpholine and 2 equiv DBU. Initially this resulted in a 1:1 mixture of the product and 7-carboxy-pterin, which was sonicated in a dilute aqueous solution of NaHCO₃ to dissolve the carboxylate, and the product was collected by filtration, resulting in 28 mg (44%) 15 after drying. MP > 300°C. ¹H NMR (400 MHz, DMSO-d₆) δ(ppm)= 11.64 (s, 1H), 8.49 (s, 1H), 7.07 (br, 2H), 3.68 (m, 4H), 3.58 (m, 2H), 3.49 (m, 2H); ¹³C NMR (100 MHz, DMSO-d₆) δ(ppm)= 164.9, 161.2, 156.3, 155.2, 152.8, 137.9, 130.1, 66.7, 66.4, 47.4, 42.5; IR (cm⁻¹): 3438, 3336, 3084, 2986, 2934, 1720, 1632, 1595, 1204; HRMS-ESI (m/z) [M+H]+ calc. for (C₁₁H₁₀N₃O₃)⁺ 277.1044; found 277.1043

2-Amino-N-benzyl-N-methyl-4-oxo-3,4-dihydropteridine-7-carboxamide (16): Synthesis of 16 required a modified method, where 0.3 mL (10 equiv) of N-methylbenzylamine was added to the solution of 50 mg 7CMP (1) in 1 mL MeOH and 2 equiv of DBU. The reaction vessel was sealed and heated to 130 °C for 3 h. Work-up proceeded as described in the general method, providing 44 mg (63%) of 16, existing as an equal mixture of the s-cis and s-trans rotamers. MP > 300°C. ¹H NMR (400 MHz, DMSO-d₆) δ(ppm)= {two rotamers} ¹¹.57 {overlapping rotamer} (s, 1H), 8.53 {8.49} (s, 1H), 7.42-7.27 {overlapping rotamer} (m, 5H), 7.03 {overlapping rotamer} (br, 2H), 4.72 {4.58} (s, 2H), 2.91 {2.90} (s, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ(ppm)= {two rotamers} 166.7 {166.5}, 161.1 {overlapping}, 155.2 {overlapping}, 153.6 {153.5}, 137.7 {137.6}, 137.2 {137.1}, 130.1 {130.0}, 129.1(2C) {129.0}, 128.9 {128.8}, 128.1(2C) {127.9}, 127.8 {overlapping}, 53.7 {50.4}, 36.6 {32.9}; IR (cm⁻¹): 3150, 2744, 1696, 1658, 1512, 1407, 1334, 777, 637; HRMS-ESI (m/z) [M+H]+ calc. for (C₁₅H₁₅N₂O₂)⁺ 311.1251; found 311.1251
**Pseudo-first order kinetics NMR data collection:**

Kinetics for the DBU-amidation was followed by $^1$H NMR for a representative set of amines. Pseudo-first order conditions were used, whereby the amine was in large excess and its change in concentration was negligible. Therefore, the rate could be viewed as:

$$\text{Rate} = k_{\text{obs}}[7-\text{CMP}]$$

Therefore, the integrated rate law becomes

$$\ln[7-\text{CMP}]_t = \ln[7-\text{CMP}]_0 + k_{\text{obs}}t,$$

and plotting $\ln[7-\text{CMP}]$ vs $t$ gives a slope of $k_{\text{obs}}$. The $k$ value for the 2nd order rate is thus determined by dividing $k_{\text{obs}}$ by the initial amine concentration.

A typical $^1$H NMR experiment begins by suspending 7-CMP in anhydrous methanol-$d_4$, followed by the addition of DBU and a large excess of the amine to provide a homogeneous solution. This solution is then transferred to an NMR tube, and a spectrum is collected every 60 min for 4–8 h. For faster reactions, data was collected every 20 min for the first hour. As glycine gave a particularly rapid reaction, data was collected every 10 min for the first 2 h. The disappearance of the aromatic C$_6$-H proton of 7-CMP (appears at 8.8 ppm in methanol-$d_4$) was coupled with the growth of the aromatic C$_6$-H proton for the amide product (typically appears between 8.91–8.95 ppm in methanol-$d_4$). Ratios of integrals were converted to $\%$ (7-CMP) and $\%$ (product), which was in turn converted to [7-CMP].

**Figure S1:** Representative NMR data, showing the shift in the pterin C$_6$-H signal as 7-CMP reacts with glycine from $t = 5$ min (top) to $t = 75$ min (bottom) in methanol-$d_4$. 
Copies of $^1$H and $^{13}$C spectra: 2 (in DMSO-$d_6$)
4 (in DMSO-\textit{d}_6)
5 (in DMSO-$d_6$)
6 (in DMSO-$d_6$)
8 (in DMSO-d6)
10 (in DMSO-d$_6$)
12 (in DMSO-d$_6$)
14 (in DMSO-\textit{d}_6)
15 (in DMSO-$d_6$)
References:
2) J.M. Pruet, J.D. Robertus, E.V. Anslyn, Tetrahedron Lett. 51 2010 2539
4) This way of indicating amide rotamers in NMR was used in the following report: X. Li; S. Danishefsky, J. Am. Chem. Soc. 130 2008, 5446